

CLAIMS

1. An  $\alpha$ -glucan phosphorylase having improved  
thermostability, which is obtained by modifying a natural  
5  $\alpha$ -glucan phosphorylase,

wherein the natural  $\alpha$ -glucan phosphorylase is derived  
from a plant, and

the  $\alpha$ -glucan phosphorylase having improved  
thermostability has an amino acid residue which is different  
10 from that of the natural  $\alpha$ -glucan phosphorylase in at least  
one position selected from the group consisting of:

a position corresponding to position 4 in a motif  
sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding  
to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;

15 a position corresponding to position 4 in a motif  
sequence 2: A-L-G-N-G-G-L-G;-and-

a position corresponding to position 7 in a motif  
sequence 3L: R-I-V-K-F-I-T-D-V or a position corresponding  
to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V;  
20 and wherein

enzyme activity of the  $\alpha$ -glucan phosphorylase having  
improved thermostability at 37°C, after heating in a 20 mM  
citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or  
more of enzyme activity of the  $\alpha$ -glucan phosphorylase having  
25 improved thermostability at 37°C, before heating.

2. The  $\alpha$ -glucan phosphorylase having improved  
thermostability according to claim 1, wherein the natural  
 $\alpha$ -glucan phosphorylase has an amino acid residue which is  
30 different from an amino acid residue of the natural  $\alpha$ -glucan  
phosphorylase at a position corresponding to position 4 in  
the motif sequence 1L, or a position corresponding to position  
4 in the motif sequence 1H, a position corresponding to

position 7 in the motif sequence 3L, or a position corresponding to position 7 in the motif sequence 3H.

5 3. The  $\alpha$ -glucan phosphorylase having improved  
thermostability according to claim 1, wherein the amino acid  
sequence of the natural  $\alpha$ -glucan phosphorylase has at least  
50% identity with an amino acid sequence selected from the  
group consisting of: a position 1 to position 916 of SEQ  
ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position  
10 1 to position 893 of SEQ ID NO: 6; position 1 to position  
939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID  
NO: 10; position 1 to position 971 of SEQ ID NO: 12; position  
1 to position 983 of SEQ ID NO: 14; position 1 to position  
928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID  
15 NO: 18; position 1 to position 832 of SEQ ID NO: 20; position  
1 to position 840 of SEQ ID NO: 22; position 1 to position  
841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID  
NO: 26; position 1 to position 841 of SEQ ID NO: 28; and  
a position 1 to position 838 of SEQ ID NO: 30.

20 4. The  $\alpha$ -glucan phosphorylase having improved  
thermostability according to claim 1, wherein the amino acid  
sequence of the natural  $\alpha$ -glucan phosphorylase is encoded  
by a nucleic acid molecule which hybridizes under stringent  
25 condition to a nucleic acid molecule consisting of a base  
sequence encoding an amino acid sequence selected from the  
group consisting of: a position 1 to position 916 of SEQ  
ID NO: 2; position 1 to position 912 of SEQ ID NO: 4; position  
1 to position 893 of SEQ ID NO: 6; position 1 to position  
30 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID  
NO: 10; position 1 to position 971 of SEQ ID NO: 12; position  
1 to position 983 of SEQ ID NO: 14; position 1 to position  
928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID

NO: 18; position 1 to position 832 of SEQ ID NO: 20; position  
1 to position 840 of SEQ ID NO: 22; position 1 to position  
841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID  
NO: 26; position 1 to position 841 of SEQ ID NO: 28; and  
5 position 1 to position 838 of SEQ ID NO: 30.

5. The  $\alpha$ -glucan phosphorylase having improved  
thermostability according to claim 1, wherein the natural  
 $\alpha$ -glucan phosphorylase is a type L  $\alpha$ -glucan phosphorylase,  
10 and has an amino acid residue which is different from that  
of the natural  $\alpha$ -glucan phosphorylase in at least one position  
selected from the group consisting of: a position  
corresponding to position 4 in the motif sequence 1L; a  
position corresponding to position 4 in the motif sequence;  
15 2 and a position corresponding to position 7 in the motif  
sequence 3L.

6. The  $\alpha$ -glucan phosphorylase having improved  
thermostability according to claim 1, wherein the natural  
20  $\alpha$ -glucan phosphorylase is a type H  $\alpha$ -glucan phosphorylase,  
and has an amino acid residue which is different from that  
of the natural  $\alpha$ -glucan phosphorylase in at least one position  
selected from the group consisting of: a position  
corresponding to position 4 in the motif sequence 1H; a  
25 position corresponding to position 4 in the motif sequence  
2; and a position corresponding to position 7 in the motif  
sequence 3H.

7. The  $\alpha$ -glucan phosphorylase having improved  
30 thermostability according to claim 1, wherein an amino acid  
sequence of the natural  $\alpha$ -glucan phosphorylase is selected  
from the group consisting of: position 1 to position 916  
of SEQ ID NO: 2; position 1 to position 912 of SEQ ID NO:

4; position 1 to position 893 of SEQ ID NO: 6; position 1 to position 939 of SEQ ID NO: 8; position 1 to position 962 of SEQ ID NO: 10; position 1 to position 971 of SEQ ID NO: 12; position 1 to position 983 of SEQ ID NO: 14; position 1 to position 928 of SEQ ID NO: 16; position 1 to position 951 of SEQ ID NO: 18; position 1 to position 832 of SEQ ID NO: 20; position 1 to position 840 of SEQ ID NO: 22; position 1 to position 841 of SEQ ID NO: 24; position 1 to position 842 of SEQ ID NO: 26; position 1 to position 841 of SEQ ID NO: 28; and position 1 to position 838 of SEQ ID NO: 30.

8. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein the natural  $\alpha$ -glucan phosphorylase is derived from potato or *Arabidopsis thaliana*.

9. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, which has an amino acid residue which is different from an amino acid residue of the natural  $\alpha$ -glucan phosphorylase in at least two positions selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H.

10. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, which has an amino acid residue which is different from an amino acid residue of the natural  $\alpha$ -glucan phosphorylase in: a position corresponding to position 4 in the motif sequence 1L or a

position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H.

11. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H is selected from the group consisting of I, L and V.

12. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H is selected from the group consisting of I and L.

13. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 2 is selected from the group consisting of A, C, D, E, G, H, I, L, M, F, S, T, V and Y.

14. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 4 in the motif sequence 2 is selected from the group consisting of C, G, S and V.

15. The  $\alpha$ -glucan phosphorylase having improved

thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H is selected from the group consisting of C, I, L, V and W.

16. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein an amino acid residue at a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H is selected from the group consisting of C, I, L and V.

17. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein enzyme activity at 37°C of the  $\alpha$ -glucan phosphorylase having improved thermostability after heated in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes is 30% or more of enzyme activity at 37°C of the  $\alpha$ -glucan phosphorylase having improved thermostability, before the heating.

18. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 65°C for 2 minutes, is 10% or more of enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, before heating.

19. The  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, wherein storage stability thereof is improved as compared with the natural  $\alpha$ -glucan phosphorylase.

20. A method for producing  $\alpha$ -glucan phosphorylase having improved thermostability, comprising:

5       modifying a first nucleic acid molecule comprising a base sequence encoding a first  $\alpha$ -glucan phosphorylase to obtain a second nucleic acid molecule comprising a modified base sequence;

      making an expression vector comprising the second nucleic acid molecule;

10       introducing the expression vector into a cell to express an  $\alpha$ -glucan phosphorylase having improved thermostability; and

      recovering the expressed  $\alpha$ -glucan phosphorylase having improved thermostability,

15       wherein the first  $\alpha$ -glucan phosphorylase is derived from a plant,

      the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the first  $\alpha$ -glucan phosphorylase in at least one position selected from the group consisting of:

      a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;

25       a position corresponding to position 4 in a motif sequence 2: A-L-G-N-G-G-L-G; and

      a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V;

30       and

      wherein the enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is

20% or more of enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, before heating.

21. The method according to claim 20, wherein an amino acid residue of the  $\alpha$ -glucan phosphorylase having improved thermostability at: a position corresponding to position 4 in the motif sequence 1L or a position corresponding to position 4 in the motif sequence 1H; or a position corresponding to position 7 in the motif sequence 3L or a position corresponding to position 7 in the motif sequence 3H; is different from an amino acid residue of the first  $\alpha$ -glucan phosphorylase.

22. The method according to claim 20, wherein the first  $\alpha$ -glucan phosphorylase is a type L  $\alpha$ -glucan phosphorylase, and has an amino acid residue which is different from that of the natural  $\alpha$ -glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1L; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3L.

23. The method according to claim 20, wherein the first  $\alpha$ -glucan phosphorylase is a type H  $\alpha$ -glucan phosphorylase, and has an amino acid residue which is different from that of the natural  $\alpha$ -glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to position 4 in the motif sequence 1H; a position corresponding to position 4 in the motif sequence 2; and a position corresponding to position 7 in the motif sequence 3H.



24. The method according to claim 20, wherein the first  $\alpha$ -glucan phosphorylase is derived from potato or *Arabidopsis thaliana*.

5     25. A nucleic acid molecule comprising a base sequence encoding the  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1.

10     26. A vector comprising the nucleic acid molecule according to claim 25.

27. A cell comprising the nucleic acid molecule according to claim 25.

15     28. A method of synthesizing a glucan, comprising reacting a reaction solution containing the  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, a sucrose phosphorylase, sucrose, a primer, and inorganic phosphoric acid or glucose-1-phosphate to produce a glucan.

20     29. The method according to claim 28, wherein the reaction is performed at a temperature of 60°C to 75°C.

25     30. A method of synthesizing a glucan, comprising reacting a reaction solution containing the  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 1, a primer, and glucose-1-phosphate to produce a glucan.

30     31. The method according to claim 30, wherein the reaction is performed at a temperature of 60°C to 75°C.

32. A method of synthesizing glucose-1-phosphate, comprising reacting a reaction solution containing  $\alpha$ -glucan

phosphorylase having improved thermostability according to claim 1, a glucan and inorganic phosphoric acid to produce glucose-1-phosphate.

5      33. The method according to claim 32, wherein the reaction is performed at a temperature of 60°C to 75°C.

34. An  $\alpha$ -glucan phosphorylase having improved thermostability, which is obtained by modifying a  
10      plant-derived natural  $\alpha$ -glucan phosphorylase,

         wherein the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the natural  $\alpha$ -glucan phosphorylase at;

15              a position corresponding to position 4 in a motif sequence 1L: H-A-E-F-T-P-V-F-S or a position corresponding to position 4 in a motif sequence 1H: H-A-Q-Y-S-P-H-F-S;  
                 a position corresponding to position 4 in a motif sequence 2L: A-L-G-N-G-G-L-G; and

20              a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V or a position corresponding to position 7 in a motif sequence 3H: R-I-V-K-L-V-N-D-V;

         wherein the enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, after heating in  
25      a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, before heating, and

         wherein the  $\alpha$ -glucan phosphorylase having improved thermostability has ability to synthesize an amylose having  
30      a weight average molecular weight of 600 kDa or more.

35. An  $\alpha$ -glucan phosphorylase having improved thermostability, which is obtained by modifying a natural

$\alpha$ -glucan phosphorylase,

wherein the natural  $\alpha$ -glucan phosphorylase is derived from a plant,

the  $\alpha$ -glucan phosphorylase having improved  
5 thermostability has an amino acid residue which is different from that of the natural  $\alpha$ -glucan phosphorylase in at least one position selected from the group consisting of a position corresponding to phenylalanine at position 39 (F39), a position corresponding to asparagine at position 135 (N135)  
10 and a position corresponding to threonine at position 706 (T706) of an amino acid sequence of SEQ ID NO: 2, and

wherein the enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is  
15 20% or more of enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, before heating.

36. A method for producing an  $\alpha$ -glucan phosphorylase having improved thermostability, comprising:

20 modifying a first nucleic acid molecule comprising a base sequence encoding first  $\alpha$ -glucan phosphorylase to obtain a second nucleic acid molecule comprising a modified base sequence;

making an expression vector comprising the second nucleic  
25 acid molecule;

introducing the expression vector into a cell to express  $\alpha$ -glucan phosphorylase having improved thermostability; and  
recovering the expressed  $\alpha$ -glucan phosphorylase having improved thermostability,

30 wherein the first  $\alpha$ -glucan phosphorylase is derived from a plant,

the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid residue which is different

from an amino acid residue of the first  $\alpha$ -glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to phenylalanine at position 39 (F39); a position corresponding to asparagine at position 135 (N135); and a position corresponding to threonine at position 706 (T706) of an amino acid sequence of SEQ ID NO: 2, and

wherein the enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, before heating.

37. A method of synthesizing a glucan, comprising reacting a reaction solution containing; the  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 35; a sucrose phosphorylase; sucrose; a primer; and an inorganic phosphoric acid or glucose-1-phosphate; to produce a glucan.

38. A method of synthesizing a glucan, comprising reacting a reaction solution containing the  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 35, a primer, and glucose-1-phosphate, to produce a glucan.

39. A method of synthesizing glucose-1-phosphate, comprising reacting a reaction solution containing the  $\alpha$ -glucan phosphorylase having improved thermostability according to claim 35, a glucan and an inorganic phosphoric acid to produce glucose-1-phosphate.

40. An  $\alpha$ -glucan phosphorylase having improved thermostability, which is obtained by modifying a plant-derived natural  $\alpha$ -glucan phosphorylase,

wherein the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the natural  $\alpha$ -glucan phosphorylase in at least one position selected from the group consisting of: a position corresponding to phenylalanine at position 39 (F39); a position corresponding to asparagine at position 135 (N135); and a position corresponding to threonine at position 706 (T706) of an amino acid sequence of SEQ ID NO: 2,

wherein the enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, after heating in a 20 mM citrate buffer (pH 6.7) at 60°C for 10 minutes, is 20% or more of enzyme activity of the  $\alpha$ -glucan phosphorylase having improved thermostability at 37°C, before heating, and

wherein the  $\alpha$ -glucan phosphorylase having improved thermostability has ability to synthesize amylose having a weight average molecular weight of 600 kDa or more.